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Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* species and genotypes in sheep in Maryland

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Abstract

In the United Kingdom and Australia sheep have been implicated as sources of *Cryptosporidium* and *Giardia* that infect humans, but no such studies have been conducted in North America. Therefore, a study was undertaken to investigate the prevalence of these parasites in sheep on a farm in Maryland. Feces were collected from 32 pregnant ewes 1, 2, and 3 days after parturition and from each of their lambs 7, 14, and 21 days after birth. The presence of *Cryptosporidium* oocysts and *Giardia* cysts was determined by both immunofluorescence microscopy and PCR/gene sequence analysis. PCR was consistently more sensitive than microscopy. The prevalence, by PCR, of *Cryptosporidium* in ewes and lambs was 25 and 77.4%, respectively. Three species/genotypes of *Cryptosporidium* were identified: *C. parvum*, a novel *C. bovis*-like genotype, and *Cryptosporidium* cervine genotype. *Cryptosporidium parvum* and the cervine genotype have been reported worldwide in human infections. The novel *C. bovis*-like genotype is reported here for the first time. The prevalence of *Giardia* in ewes and lambs was 12 and 4%, respectively. Most infections were Assemblage E which is not zoonotic; however, one ewe was infected with zoonotic Assemblage A. The identification of only two lambs infected with *C. parvum* and one ewe infected with *G. duodenalis* Assemblage A suggests a low prevalence of these zoonoses. However, the high prevalence of the zoonotic cervine genotype indicates that sheep should be considered a potential environmental source of this human pathogen.

Keywords: *Cryptosporidium*; Genotyping; Epidemiology; *Giardia*; PCR; Sheep

1. Introduction

Cryptosporidium has been reported in a wide variety of vertebrate hosts (Fayer et al., 2000a). There is now strong evidence that there are numerous genetically distinct *Cryptosporidium* species and genotypes which are morphologically identical to *C. parvum* (Xiao et al., 2002, 2004). In cattle, for example, molecular studies have identified four species and one genotype of *Cryptosporidium* (Santín et al., 2004; Fayer et al., 2006). Although most prevalence studies of *Cryptosporidium* infection in farm animals have focused on cattle, *Cryptosporidium* has been reported in sheep worldwide (Majewska et al., 2000; McLauchlin et al., 2000; Alonso-Fresan et al., 2005; Ryan et al., 2005). In sheep, however, most prevalence information is based on microscopy and thus there is little information on *Cryptosporidium* species or genotypes. In Australia, *C. hominis*, *C. andersoni*, *C. suis*, a novel Bovine B genotype, cervine genotype, marsupial genotype, pig II genotype, and a novel previously unidentified genotype

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have been reported (Ryan et al., 2005). In the United Kingdom, *C. parvum* and a novel genotype were reported in sheep as well (Chalmers et al., 2002).

Giardia duodenalis (syn. *G. lamblia*, *G. intestinalis*) is a commonly identified intestinal parasite of mammals, including humans. *G. duodenalis* isolates from different host species are morphologically indistinguishable from each other and have been grouped into assemblages (genotypes) based on molecular characteristics (Thompson et al., 2000). Assemblages A and B infect a variety of mammals and are the only genotypes reported in humans. Assemblages C through G appear to be host-specific (Monis et al., 1999, 2003; Sulaiman et al., 2003; Caccio et al., 2005). *Giardia* infection in sheep is relatively common and has been reported worldwide (Xiao et al., 1994; Diaz et al., 1996; Giangaspero et al., 2005; Ryan et al., 2005; Ozmen et al., 2006; van der Giessen et al., 2006). Three genotypes of *G. duodenalis* have been identified in sheep, Assemblage E, the livestock genotype and also the two zoonotic genotypes, Assemblages A and B (Giangaspero et al., 2005; Ryan et al., 2005; Aloisio et al., 2006; van der Giessen et al., 2006).

Cryptosporidium oocysts and *Giardia* cysts excreted in the feces of infected animals can be a source of human infection; therefore it is essential to confirm the species or genotypes of theses parasites that are present in infected animals. Few molecular studies of *Cryptosporidium* and *Giardia* in sheep have been conducted and none of these studies has included sheep from North America. The present study was conducted to assess the prevalence of these parasites in a flock of sheep in Maryland, USA, and through DNA sequence analysis, to identify the species and genotypes that were present.

2. Material and methods

2.1. Sources and collection of specimens

Thirty-two ewes and 31 lambs, located on a farm in Carroll County, Maryland, were included in this study; all were of the Hampshire breed. Fecal samples from ewes were collected for 3 consecutive days after parturition, and samples from lambs born to those ewes were collected at 7, 14, and 21 days of age.

Ewes ranged in age from 2 to 6 years old and were housed in groups in a barn. Feces were collected directly from the rectum of each ewe and lamb into a plastic specimen cup that was immediately capped, labeled, and placed on ice in an insulated container. Feces were transported to the USDA laboratory in Beltsville, MD, and processed within 1–3 days of collection.

2.2. Cleaning of specimens from feces

Feces from ewes were processed as previously described (Fayer et al., 2000b). Briefly, 15 g of feces from each specimen cup were transferred to a 50 ml centrifuge tube containing approximately 35 ml dH_2O. The contents of each tube were thoroughly mixed (Vortex-Genie, Scientific Industries, Bohemia, New York), passed through a 45 μm pore size screen into a second 50 ml tube, and the final volume adjusted to 50 ml with dH_2O. The tubes were centrifuged at $1800 \times g$ for 15 min, and the supernatant was discarded; the pellet was suspended in 25 ml dH_2O and mixed well by vortexing. Twenty-five milliliters of CsCl (1.4 g/l) was added to each tube, mixed thoroughly, and centrifuged at $300 \times g$ for 20 min. Four milliliters of supernatant, aspirated from the top of each suspension, was transferred to a 15 ml centrifuge tube, and dH_2O added to reach a final volume of 15 ml. Specimens were centrifuged at $1800 \times g$ for 15 min and washed twice with dH_2O before the final pellet was suspended in 500 μl of dH_2O. Portions of the 500 μl suspension were examined by immunofluorescence microscopy and molecular analysis as described below.

The quantity of lamb feces was often insufficient for processing as describe above. In such cases, a fecal smear was prepared on a glass slide for microscopic examination and the remainder of the specimen was subjected to direct DNA extraction for molecular analysis.

2.3. Microscopic examination

For feces from ewes, a 100 μl aliquot of fecal suspension was transferred to a microcentrifuge tube and washed once with dH_2O. The pellet was resuspended in 50 μl of premixed Merifluor reagents (Meridian Diagnostics, Cincinnati, OH) and 2 μl was transferred to a three-well (11 mm diameter) glass microscope slide. For direct smears from lambs in which less than 15 g of feces were present, premixed Merifluor reagent (Meridian Diagnostics) was pipetted directly onto the surface of the smear. In all cases, the slide was covered with a 24 mm × 50 mm coverslip and examined by fluorescence microscopy at 400× using a Zeiss Axioskop equipped with epifluorescence and an FITC-Texas Red™ dual wavelength filter.

2.4. DNA extraction

Total DNA was extracted from each CsCl-cleaned fecal specimen using a DNeasy Tissue Kit (Qiagen, Valencia, CA) with a slightly modified protocol. A total

of 50 μl of fecal suspension was mixed with 180 μl of ATL buffer and thoroughly mixed by vortexing. To this suspension, 20 μl of proteinase K (20 mg/ml) was added, and the sample was thoroughly mixed. Following an overnight incubation at 55 °C, 200 μl of AL buffer was added. The remainder of the protocol followed the manufacturer's instructions with one exception: to increase the quantity of recovered DNA, elution was performed with 100 μl of AE buffer.

2.5. SSU rDNA gene amplification and sequencing

A two-step nested PCR protocol was used to amplify a fragment of the SSU rRNA gene of *Cryptosporidium* (~830 bp). Amplification was performed using primers previously described (Xiao et al., 1999). For the primary PCR step, the reaction contained 1× PCR buffer, 3 mM $MgCl_2$, 0.2 mM each dNTP, 2.5 U Taq, 2.5 μl of BSA (0.1 g/10 ml), and 1 μM of each primer in a total reaction volume of 50 μl. A total of 35 cycles, each consisting of 94 °C for 45 s, 59 °C for 45 s, and 72 °C for 1 min, were performed; an initial hot start at 94 °C for 3 min and a final extension step at 72 °C for 7 min were also included. The nested PCR mixture was identical to the primary PCR except that a concentration of 1.5 mM $MgCl_2$ was used. The cycling conditions were as follows: 40 cycles of 94 °C for 30 s, 58 °C for 90 s, and 72 °C for 2 min, with an initial hot start at 94 °C for 3 min and a final extension step at 72 °C for 7 min.

A fragment of the SSU rDNA for *Giardia* (~292 bp) gene was amplified by PCR using primers previously described (Hopkins et al., 1997). For the primary PCR step, the PCR mixture contained 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 2 U Taq, 2.5 μl of dimethyl sulfoxide (DMSO), and 0.5 μM of each primer in a total reaction volume of 50 μl. The PCR conditions were as follows: 35 cycles of 96 °C for 45 s, 58 °C for 30 s, and 72 °C for 45 s, with an initial hot start at 96 °C for 2 min and a final extension step at 72 °C for 4 min. The nested PCR mixture was identical to that of the primary PCR. The cycle conditions for the nested PCR were identical to the primary PCR annealing temperature, which was lowered to 55 °C.

The PCR products were subjected to electrophoresis in a 1% agarose gel and visualized by staining with ethidium bromide. Negative and positive controls were included in all PCR sets.

2.6. DNA sequence analysis

PCR products were treated with Exonuclease I/shrimp alkaline phosphatase (Exo-SAP-IT™) (USB Corporation, Cleveland, OH) and sequenced in both directions. Sequencing reactions used the same primers used for PCR, Big Dye™ Chemistry, and an ABI3100 sequencer analyzer (Applied Biosystems, Foster City, CA). Sequence chromatograms from each strand were aligned using Lasergene software (DNASTAR, Inc., Madison, WI).

2.7. Statistic analysis

Fisher's exact test was performed using GraphPad InStat version 3.06 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

3. Results

3.1. Prevalence of Cryptosporidium and Giardia by IFA and PCR

PCR was a more sensitive detection method than immuno-fluorescence microscopy. In a comparison of all 189 samples collected, *Cryptosporidium* was detected in 30% by PCR versus only 9% by microscopy (Fisher's exact test, $P < 0.0001$), and *Giardia* was detected in 11.6% PCR versus 6% by microscopy (Fisher's exact test, $P = 0.0671$).

The prevalence of *Cryptosporidium* and *Giardia* in ewes and lambs as determined by IFA and PCR is shown in Table 1. Microscopic analysis of 3 fecal specimens for each of the 63 animals included in the study revealed

Table 1
Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in ewes and lambs by PCR and IFA

	No. of animals examined	No. of positives (prevalence)			
		Cryptosporidium spp.		*Giardia* spp.	
		IFA	PCR	IFA	PCR
Ewes	32	3 (9.4)	8 (25)	6 (18.75)	12 (37.5)
Lambs	31	10 (32.25)	24 (77.4)	2 (6.45)	4 (12.9)
Total	63	13 (20.6)	32 (50.8)	8 (12.7)	16 (25.4)

Values in parenthesis denote percentage.

Table 2
Cryptosporidium and *Giardia* species/genotypes identified in ewes and lambs

ID#	Ewe		Lamb	
	Cryptosporidium	*Giardia*	*Cryptosporidium*	*Giardia*
1	Cervine 1 (1, 2, 3)	–	Ovine (14)	–
2	Cervine 1 (2, 3)	–	Cervine 2 (21)	Assemblage E (7, 14, 21)
3	–	–	Ovine (14, 21)	–
4	–	–	Cervine 3 (21)	Assemblage E (7)
5	–	–	–	–
6	–	–	Cervine 1 (14, 21)	–
7	–	–	Cervine 1 (14, 21)	–
8	–	–	–	–
9	–	–	–	–
10	Cervine 2 (1)	–	–	–
11	*C. parvum* (3)	–	–	–
12	–	–	Cervine 1 (21)	–
13	–	Assemblage E (2)	–	–
14	–	–	–	–
15	–	–	Cervine 1 (21)	–
16	–	Assemblage E (2)	Cervine 1 (7) Ovine (21)	–
17	Cervine 1 (1, 2, 3)	Assemblage E (1, 3)	Cervine 2 (14)	–
18	Ovine (1, 2, 3)	Assemblage E (2)	N/A	N/A
19	–	Assemblage E (2, 3)	Cervine 1 (7, 21)	Assemblage E (21)
20	–	–	Cervine 3 (14, 21)	–
21	–	–	Cervine 1 (7, 14, 21)	–
22	Cervine 1 (2, 3)	Assemblage E (1, 3)	Cervine 1 (7, 14)	Assemblage E (14,21)
23	–	–	Cervine 3 (14)	–
24	–	–	Cervine 2 (14)	–
25	–	–	Cervine 2 (14, 21)	–
26	–	Assemblage A (3)	Cervine 2 (7, 14, 21)	–
27	–	–	Cervine 2 (21)	–
28	Cervine 1 (3)	Assemblage E (1, 2, 3)	*C. parvum* KSU (7) Cervine 1 (14, 21)	–
29	–	–	Cervine 3 (14, 21)	–
30	–	Assemblage E (2)	Cervine 1 (21)	–
31	–	Assemblage E (1)	Cervine 1 (21)	–
32	–	–	Cervine 1 (7, 14, 21)	–

Each ewe is paired in the same row of the table with its lamb. In parenthesis is the day of collection of the positive specimens, in ewes for days 1, 2, and 3 after parturition and in lambs for days 7, 14, and 21 after birth. N/A: not available (lamb died during the study).

that 13 (20.6%) and 8 (12.7%) were shedding oocysts/cysts of *Cryptosporidium* and *Giardia*, respectively. PCR analysis of the same samples revealed that 32 (50.8%) and 16 (25.4%) were shedding oocysts/cysts of *Cryptosporidium* and *Giardia*, respectively. The higher prevalence found by PCR was significant for *Cryptosporidium* (Fisher's exact test, $P = 0.0007$) but not for *Giardia* (Fisher's exact test, $P = 0.1110$). Mixed infections with *Cryptosporidium* and *Giardia* were detected in four ewes and four lambs (Table 2).

The prevalence of *Cryptosporidium* varied considerably between ewes and lambs, with a higher prevalence in lambs (Fisher's exact test: IFA, $P = 0.0319$, and PCR, $P < 0.0001$) (Table 1). In contrast, the prevalence of *Giardia* was higher in ewes than in lambs but the difference was significant only for IFA (Fisher's exact test: IFA, $P = 0.0413$, and PCR, $P = 0.2565$) (Table 1).

3.2. Molecular characterization of *Cryptosporidium* isolates by PCR and SSU rRNA gene sequencing

All 189 fecal samples collected (3 samples for each of 32 ewes and 31 lambs) were screened for *Cryptosporidium* by PCR at the SSU rDNA locus (Table 2).

Fifty-seven specimens corresponding to 8 ewes and 24 lambs were positive for *Cryptosporidium* (Table 2).

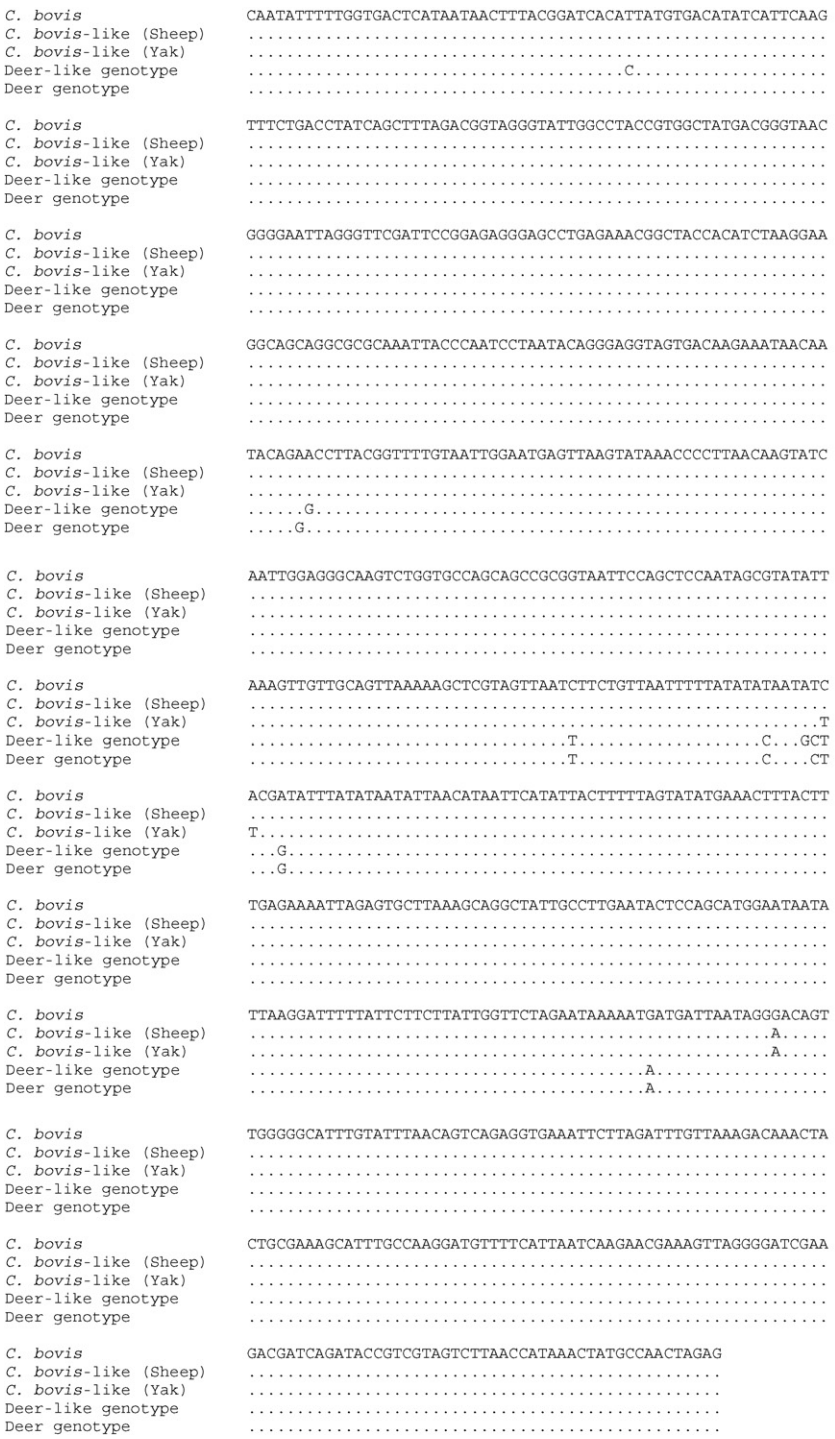

Fig. 1. Differences in the 769 bp fragment of the SSU rRNA gene among *C. bovis*, *C. bovis*-like (yak), *C. bovis*-like (sheep), deer genotype and deer-like genotype. Dots denote nucleotide identity to *C. bovis* sequence (AY741305). Only the sequences of the polymorphic region are shown. Compared to *C. bovis*, there is only one nucleotide change in the *C. bovis*-like (sheep) sequence.

The number of ewes shedding *Cryptosporidium* on days 1, 2, and 3 after parturition was 4, 5, and 7. The number of lambs shedding *Cryptosporidium* at days 7, 14, and 21 after birth was 7, 15, and 19.

Sequence analysis of all 57 specimens positive by PCR identified *C. parvum*, *Cryptosporidium* cervine genotype, and *C. bovis*-like in 2, 48, and 7 specimens, respectively (Table 2). In two lambs, more than one genotype of *Cryptosporidium* was detected. One lamb had the cervine genotype and *C. bovis*-like and the other lamb had *C. parvum* and the cervine genotype (Table 2).

Two types of *C. parvum* sequences (AF093490 and AF308600), and three types of *Cryptosporidium* cervine genotype sequences (cervine 1–3) were identified. Specimens in the present study demostrated 100% similarity to the *Cryptosporidium* cervine 1 (AF442484) and 2 (AY458613) sequences previously reported. The detection of a third cervine genotype sequence, which we designate as cervine 3, is reported here for the first time. The sequence diversity was 0.2% between cervine 3 and 1 and 0.3% between cervine 3 and 2. *Cryptosporidium* cervine 3 exhibited a nucleotide variation at position 587 (nucleotide A instead of nucleotide T) when compared with cervine 1. Another novel *Cryptosporidium* was found in the present study, and we designate it as *C. bovis*-like. This *C. bovis*-like sequence shared 99.9% similarity with *C. bovis* (AY741305), 99% with *Cryptosporidium* deer genotype (AY120910) and 98.7% similarity with the deer-like genotype (AY587166) (Fig. 1). The nucleotide sequences of the SSU rDNA gene of *Cryptosporidium* obtained in this study have been deposited in GenBank under accession numbers EF362478–EF362481.

3.3. Molecular characterization of Giardia isolates by PCR and SSU rRNA gene sequencing

Giardia was detected by PCR analysis in 22 of 189 fecal samples (3 samples for each of 32 ewes and 31 lambs) corresponding to 12 ewes and 4 lambs (Table 2). The number of ewes shedding *Giardia* at days 1, 2, and 3 after parturition was 4, 6, and 5, respectively (Table 2). The number of lambs shedding *Giardia* at days 7, 14, and 21 after birth was 2, 2, and 3, respectively (Table 2).

Sequence analysis of all 22 *Giardia* positive fecal specimens identified 21 specimens as Assemblage E (AY655701), and 1 specimen, from an ewe, as Assemblage A (AY655700) (Table 2).

4. Discussion

Most previous studies on *Cryptosporidium* and *Giardia* in sheep were based on microscopy and reported prevalences ranging from 2.7 to 38% for *Giardia* (Olson et al., 1997; Causape et al., 2002) and 2.6 to 82% for *Cryptosporidium* (Abd-El-Wahed, 1999; Ryan et al., 2005). In the present study, PCR detection was more sensitive than microscopy, a finding reported in other studies as well (Chalmers et al., 2002; Ryan et al., 2005); *Cryptosporidium* and *Giardia* were detected in 3× and 2× more specimens, respectively by PCR than by microscopy. Fecal shedding of these parasites often occurs sporadically and in low numbers, especially in asymptomatic carriers; therefore a sensitive technique, such as PCR, should be used when fecal samples are examined. Our results also show that the prevalence of *Giardia* and *Cryptosporidium* could be underestimated by both molecular and microscopic methods when only one fecal specimen is collected per animal (Table 2). A negative specimen would indicate that an animal was not infected when there might actually be a pattern of intermittent oocyst/cyst excretion.

Lambs were more often infected with *Cryptosporidium* than adult sheep; this age related difference has been reported previously (Xiao et al., 1993; Causape et al., 2002; Majewska et al., 2000; Ryan et al., 2005). The highest prevalence of *Cryptosporidium* among the lambs was seen at 21 days of age when 19 of 31 lambs were shedding *Cryptosporidium*. In other studies *Cryptosporidium* was shown to peak in lambs less than 14 days of age (Xiao et al., 1993; Abd-El-Wahed, 1999; Causape et al., 2002).

The prevalence of *Giardia* was higher in ewes than in lambs. This contrasts with most studies wherein more lambs were reported to be infected with *Giardia* than adults (Olson et al., 1997; Ryan et al., 2005). One explanation for this difference might be that in other studies, lambs were defined as being less than 1 year of age whereas in our study all lambs were less than 21 days of age.

Cryptosporidiosis and giardiasis are major public health concerns. However, the risk of zoonotic infection from sheep cannot be determined without more extensive, genetically defined prevalence data. The present study suggests that sheep are more likely to be infected with the *Cryptosporidium* cervine genotype than with *C. parvum*. Likewise, in Australia the cervine genotype was also the most prevalent (33/57 specimens), and *C. parvum* was not identified in any of the 60 isolates sequenced from sheep (Ryan et al., 2005). Most studies in which *C. parvum* was reported in sheep were based solely on microscopy, without molecular characterization. Considering those reports in light of recent advances in knowledge and methodology, it is not

possible to know what species/genotypes were actually present. The cervine genotype has both a wide host and geographic range, having been found in sheep in Australia, lemurs and white-tailed deer in North America, blesbok, mouflon, and nyala in the Czech Republic, and in humans in Canada, New Zealand, the United Kingdom, Slovenia, and the United States (Perz and Le Blancq, 2001; Ong et al., 2002; da Silva et al., 2003; Ryan et al., 2003, 2005; Learmonth et al., 2004; Blackburn et al., 2006; Feltus et al., 2006; Leoni et al., 2006; Soba et al., 2006), thus, this genotype could emerge as an important zoonotic pathogen.

A novel *Cryptosporidium* was found in four sheep. Because of the high sequence similarity (99.9%) with *C. bovis*, it was assigned the name, *C. bovis*-like (Fig. 1). A high sequence similarity has also been seen between *C. bovis* from cattle and a *C. bovis*-like isolate from a yak in China (Feng et al., 2007). Our findings increase the number of *Cryptosporidium* genotypes, which now exceed 30, and attest to the great genetic diversity within this genus. Many of these genotypes may eventually be elevated to species level classification as more data accrue on unique biological and other characteristics. For example, in cattle, *C. bovis* (previously known as Bovine B) and the *Cryptosporidium* deer-like genotype are now recognized as host-specific (Santín et al., 2004; Fayer et al., 2005). Two other novel genotypes in sheep were identified in previous studies (Chalmers et al., 2002; Ryan et al., 2005). However, it was not possible to compare the sequences found in sheep by Chalmers et al. (2002) because no sequence data were reported and also a different locus was sequenced. Attempts to amplify the *Cryptosporidium* oocysts wall protein gene (COWP gene) (per Spano et al., 1997) with specimens identified as *C. bovis*-like were unsuccessful (data not shown). The other novel genotype identified in sheep by Ryan et al. (2005) (AY898790) was different from the *C. bovis*-like genotype found in the present study.

Sequence analysis of 22 *Giardia* isolates identified 21 as Assemblage E (21 isolates) and only 1 isolate as Assemblage A. Assemblage E is commonly found in hoofed animals including cattle and sheep (Trout et al., 2004; Ryan et al., 2005). Assemblage A has been identified in humans as well as in sheep and other animals (Thompson et al., 2000; Trout et al., 2004; Ryan et al., 2005).

5. Conclusion

This study demonstrates that sheep are capable of harboring *Cryptosporidium* and *Giardia* species and genotypes that are known to be zoonotic as well as those that appear to be host-specific. In addition, a *C. bovis*-like isolate was reported from sheep for the first time. Clear differences in the prevalence of *Cryptosporidium* and *Giardia* were found based on the age of the animals that were examined, with more neonates having cryptosporidiosis and fewer neonates having giardiasis. Because this study dealt with a single farm, a more comprehensive understanding of the overall prevalence of these parasites will emerge as molecularly based epidemiologic studies are undertaken on more farms and over a wider geographic range.

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References

Abd-El-Wahed, M.M., 1999. *Cryptosporidium* infection among sheep in Qalubia Governorate, Egypt. J. Egypt. Soc. Parasitol. 29, 113–118.

Aloisio, F., Filippini, G., Antenucci, P., Lepri, E., Pezzotti, G., Caccio, S.M., Pozzio, E., 2006. Severe weight loss in lambs infected with *Giardia duodenalis* Assemblage B. Vet. Parasitol. 142, 154–158.

Alonso-Fresan, M.U., Garcia-Alvarez, A., Salazar-Garcia, F., Vazquez-Chagoyan, J.C., Pesacador-Salas, N., Saltijeral-Oaxaca, J., 2005. Prevalence of *Cryptosporidium* spp. in asymptomatic sheep in family flocks from Mexico State. J. Vet. Med. 52, 482–483.

Blackburn, B.G., Mazurek, J.M., Hlavsa, M., Park, J., Tillapaw, M., Parrish, M., Salehi, E., Franks, W., Koch, E., Smith, F., Xiao, L., Arrowood, M., Hill, V., da Silva, A., Johnston, S., Jones, J.L., 2006. Cryptosporidiosis associated with ozonated apple cider. Emerg. Infect. Dis. 12, 684–686.

Caccio, S.M., Thompson, R.C.A., McLauchlin, J., Smith, H.V., 2005. Unravelling *Cryptosporidium* and *Giardia* epidemiology. Trends Parasitol. 21, 430–437.

Causape, A.C., Quilez, J., Sanchez-Acedo, C., del Cacho, E., Lopez-Bernad, F., 2002. Prevalence and analysis of potential risk factors for *Cryptosporidium parvum* infection in lambs in Zaragoza (northeastern Spain). Vet. Parasitol. 104, 287–298.

Chalmers, R.M., Elwin, K., Reilly, W.J., Irvine, H., Thomas, A.L., Hunter, P.R., 2002. *Cryptosporidium* in farmed animals: the detection of a novel isolate in sheep. Int. J. Parasitol. 32, 21–26.

da Silva, A.J., Caccio, S., Williams, C., Won, K.Y., Nace, E.K., Whittier, C., Pieniazek, N.J., Eberhard, M.L., 2003. Molecular and morphologic characterization of a *Cryptosporidium* genotype identified in lemurs. Vet. Parasitol. 111, 297–307.

Diaz, V., Campos, M., Lozano, J., Manas, I., Gonzalez, J., 1996. Aspects of animal giardiosis in Granada province (southern Spain). Vet. Parasitol. 64, 171–176.

Fayer, R., Morgan, U., Upton, S.J., 2000a. Epidemiology of *Cryptosporidium*: transmission, detection and identification. Int. J. Parasitol. 30, 1305–1322.

Fayer, R., Trout, J.M., Craczyk, T.D., Lewis, E.J., 2000b. Prevalence of *Cryptosporidium*, *Giardia*, and *Eimeria* infections in post-weaned

and adult cattle on three Maryland farms. Vet. Parasitol. 93, 103–112.

Fayer, R., Santín, M., Trout, J.M., Greiner, E., 2006. Prevalence of species and genotypes of *Cryptosporidium* found in 1–2-year-old dairy cattle in the eastern United States. Vet. Parasitol. 135, 105–112.

Fayer, R., Santín, M., Xiao, L., 2005. *Cryptosporidium bovis* N. Sp. (Apicomplexa: Cryptosporidiidae) in cattle (Bos taurus). J. Parasitol. 91, 624–629.

Feltus, D.C., Giddings, C.W., Schneck, B.L., Monson, T., Warshauer, D., McEvoy, J.M., 2006. Evidence supporting zoonotic transmission of *Cryptosporidium* spp. in Wisconsin. J. Clin. Microbiol. 44, 4303–4308.

Feng, Y., Ortega, Y., He, G., Das, P., Xu, M., Zhang, X., Fayer, R., Gatei, W., Cama, V., Xiao, L., 2007. Wide geographic distribution of *Cryptosporidium bovis* and the deer-like genotype in bovines. Vet. Parasitol. 144, 1–9.

Giangaspero, A., Paoletti, B., Iorio, R., Traversa, D., 2005. Prevalence and molecular characterization of *Giardia duodenalis* from sheep in central Italy. Parasitol. Res. 96, 32–37.

Hopkins, R.M., Meloni, B.P., Groth, D.M., Wetherall, J.D., Reynoldson, J.A., Thompson, R.C., 1997. Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. J. Parasitol. 83, 44–51.

Learmonth, J.J., Ionas, G., Ebbett, K.A., Kwan, E.S., 2004. Genetic characterization and transmission cycles of *Cryptosporidium* species isolated from humans in New Zealand. Appl. Environ. Microbial. 70, 3973–3978.

Leoni, F., Amar, C., Nichols, G., Pedraza-Diaz, S., McLauchlin, J., 2006. Genetic analysis of Cryptosporidium from 2,414 humans with diarrhea in England between 1985 and 2000. J. Med. Microbiol. 55, 703–707.

Majewska, A.C., Werner, A., Sulima, P., Luty, T., 2000. Prevalence of *Cryptosporidium* in sheep and goats bred on five farms in west-central region of Poland. Vet. Parasitol. 89, 269–275.

McLauchlin, J., Amar, C., Pedraza-Diaz, S., Nichols, G.L., 2000. Molecular epidemiological analysis of *Cryptosporidium* spp. in the United Kingdom: results of genotyping *Cryptosporidium* spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. J. Clin. Microbiol. 38, 3984–3990.

Monis, P.T., Andrews, R.H., Mayrhofer, G., Ey, P.L., 1999. Molecular systematics of the parasitic protozoan *Giardia intestinalis*. Mol. Biol. Evol. 16, 1135–1144.

Monis, P.T., Andrews, R.H., Mayrhofer, G., Ey, P.L., 2003. Genetic diversity within the morphological species *Giardia intestinalis* and its relationship to host origin. Infect. Genet. Evol. 3, 29–38.

Olson, M.E., Thorlakson, C.L., Deselliers, L., Morck, D.W., McAllister, T.A., 1997. *Giardia* and *Cryptosporidium* in Canadian farm animals. Vet. Parasitol. 68, 375–381.

Ong, C.S.L., Eisler, D.L., Alikhani, A., Fung, V.W.K., Tomblin, J., Bowie, W.R., Isaac-Renton, J.L., 2002. Novel *Cryptosporidium* genotypes in sporadic cryptosporidiosis cases: first report of human infections with a cervine genotype. Emerg. Infect. Dis. 8, 263–268.

Ozmen, O., Yukari, B.A., Haligur, M., Sahinduran, S., 2006. Observations and immunohistochemical detection of *Coronavirus, Cryptosporidium parvum* and *Giardia intestinalis* in neonatal diarrhoea in lambs and kids. Schweiz. Arch. Tierheilkd. 148, 357–364.

Perz, J.F., Le Blancq, S.M., 2001. *Cryptosporidium parvum* infection involving novel genotypes in wildlife from lower New York State. Appl. Environ. Microbiol. 67, 1154–1162.

Ryan, U., Xiao, L., Read, C., Zhou, L., Lal, A.A., Pavlasek, I., 2003. Identification of novel *Cryptosporidium* genotypes from the Czech Republic. Appl. Environ. Microbiol. 69, 4302–4307.

Ryan, U.M., Bath, C., Robertson, I., Read, C., Elliot, A., Mcinnes, L., Traub, R., Besier, B., 2005. Sheep may not be an important zoonotic reservoir for *Cryptosporidium* and *Giardia* parasites. Appl. Environ. Microbiol. 71, 4992–4997.

Santín, M., Trout, J.M., Xiao, L., Zhou, L., Greiner, E., Fayer, R., 2004. Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet. Parasitol. 122, 103–117.

Soba, B., Petrovec, M., Mioc, V., Logar, J., 2006. Molecular characterization of *Cryptosporidium* isolates from humans in Slovenia. Clin. Microbiol. Infect. 12, 918–921.

Spano, F., Putignani, L., McLauchlin, J., Casemore, D.P., Crisanti, A., 1997. PCR-RFLP analysis of the *Cryptosporidium* oocyst wall protein (COWP) gene discriminates between *C. wrairi* and *C. parvum* and between *C. parvum* isolates of human and animal origin. FEMS Microbiol. Lett. 150, 207–217.

Sulaiman, I.M., Fayer, R., Bern, C., Gilman, R.H., Trout, J.M., Schantz, P.M., Das, P., Lal, A.A., Xiao, L., 2003. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg. Infect. Dis. 9, 1444–1452.

Thompson, R.C.A., Hopkins, R.M., Homan, W.L., 2000. Nomenclature and genetic groupings of *Giardia* infecting mammals. Parasitol. Today 16, 210–213.

Trout, J.M., Santín, M., Greiner, E., Fayer, R., 2004. Prevalence of *Giardia duodenalis* genotypes in pre-weaned dairy calves. Vet. Parasitol. 124, 179–186.

van der Giessen, J.W.B., de Vries, A., Roos, M., Wielinga, P., Kortbeek, L.M., Mank, T.G., 2006. Genotyping of *Giardia* in Dutch patients and animals: a phylogenetic analysis of human and animal isolates. Int. J. Parasitol. 36, 849–858.

Xiao, L., Fayer, R., Ryan, U., Upton, S.J., 2004. *Cryptosporidium* taxonomy: recent advances and implications for public health. Clin. Microbiol. Rev. 17, 72–97.

Xiao, L., Herd, R.P., McClure, K.E., 1994. Periparturient rise in the excretion of *Giardia* sp. cysts and *Cryptosporidium parvum* oocysts as a source of infection for lambs. J. Parasitol. 80, 55–59.

Xiao, L., Herd, R., Rings, D., 1993. Diagnosis of *Cryptosporidium* on a sheep farm with neonatal diarrhea by immunofluorescence assays. Vet. Parasitol. 47, 17–23.

Xiao, L., Morgan, U., Limor, J., Escalante, A., Arrowood, M., Shulaw, W., Thompson, R.C.A., Fayer, R., Lal, A., 1999. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl. Environ. Microbiol. 65, 3386–3391.

Xiao, L., Sulaiman, I.M., Ryan, U., Zhou, L., Atwill, E.R., Tischler, M.L., Zhang, X., Fayer, R., Lal, A.A., 2002. Host adaptation and host–parasite co-evolution in *Cryptosporidium*: implications for taxonomy and public health. Int. J. Parasitol. 32, 1773–1785.